Identification of a Novel ZNF469 Mutation in a Pakistani Family With Brittle Cornea Syndrome

Micheal, Shazia; Siddiqui, Sorath Noorani; Zafar, Saemah Nuzhat; Gabriëla Niewold, Ilse Therésia; Khan, Muhammad Imran; Bergen, Arthur A. B.

Published in:
Cornea

DOI:
10.1097/ICO.0000000000001828

Published: 01/01/2019

Citation for pulished version (APA):
Micheal, S., Siddiqui, S. N., Zafar, S. N., Gabriëla Niewold, I. T., Khan, M. I., & Bergen, A. A. B. (2019). Identification of a Novel ZNF469 Mutation in a Pakistani Family With Brittle Cornea Syndrome. *Cornea*, *38*(6), 718-722. https://doi.org/10.1097/ICO.0000000000001828

Identification of a Novel *ZNF469* Mutation in a Pakistani Family With Brittle Cornea Syndrome

Shazia Micheal, PhD, Sorath Noorani Siddiqui, FCPS,† Saemah Nuzhat Zafar, FCPS,† Ilse Therésia Gabriëla Niewold, BS,* Muhammad Imran Khan, PhD,‡ and Arthur A. B. Bergen, PhD**

Purpose: Brittle cornea syndrome (BCS) is a rare recessive disorder affecting connective tissues, most prominently in the eye. Pathogenic mutations causing BCS have been identified in *PRDM5* and *ZNF469* genes. This study investigates the genetic cause of BCS in a large, consanguineous Pakistani family with 4 affected and 3 unaffected individuals.

Methods: The coding region and exon–intron splice junctions of *PRDM5* and *ZNF469* genes were amplified by polymerase chain reaction, and bidirectional Sanger sequencing was performed to find the pathogenic change responsible for causing the disease in the family.

Results: A novel homozygous duplication c.9831dupC (p.Arg3278GlnfsX197) in the *ZNF469* gene was identified, which was found to be co-segregating with the disease in the family.

Conclusions: This is the first report of a *ZNF469* homozygous mutation causing a BCS phenotype in a consanguineous Pakistani family. Our data extend the mutation spectrum of *ZNF469* variants implicated in BCS.

Key Words: brittle cornea syndrome, Sanger sequencing, mutation, *ZNF469*, homozygous, duplication

(*Cornea* 2019;38:718–722)

Brittle cornea syndrome (BCS; MIM 229200) is a rare and clinically heterogeneous connective tissue disorder with an autosomal recessive mode of inheritance. The phenotype typically includes both variable ocular and extraocular features. The clinical hallmark of the disease is corneal thinning and fragility which leads to frequent corneal rupture/perforation even on minor injuries. The other common ocular features observed in patients with BCS include blue sclera, keratoconus, keratoglobus, and high myopia.[1] In patients with BCS, often visual acuity is already significantly affected by keratoconus (steep cornea with a corneal refractive power exceeding 48D) and high myopia before corneal rupture. Systemic features in patients with BCS frequently include hyperelasticity of the skin, hypermobility of the joints, kyphoscoliosis, and hearing impairment.[1–3]

BCS is also a genetically heterogeneous disease: Biallelic mutations in the *ZNF469* gene (MIM 612078) on chromosome 16q24[4–6] and in the *PRDM5* gene (MIM 614161) on chromosome 4q27[7,8] have both been associated with the disease. *ZNF469* was the first gene identified for its association with BCS, but the pathologic mechanism remains to be elucidated. Indeed, ZNF469 is a very poorly characterized single exon zinc finger protein of unknown function. PRDM5 belongs to the family of PRDM (PRDI-BF1 and RIZ domain containing) transcription factor proteins.[9]

In this article, we report a novel duplication mutation c.9831dupC (p.Arg3278GlnfsX197) in the *ZNF469* gene in a large Pakistani family with BCS having 4 affected individuals.

PATIENTS AND METHODS

Patients

In this study, a large, consanguineous Pakistani family with BCS phenotype was recruited at the pediatric department of Al-Shifa Eye Trust Hospital, Rawalpindi, Pakistan (Fig. 1A). The study was approved by the Institutional Review Board of the hospital, and it adhered to the tenets of the Declaration of Helsinki. Detailed ophthalmic examination was performed for both affected and unaffected individuals of the family; it included anterior segment examination by slit-lamp biomicroscopy with measurements of intraocular pressure by applanation tonometry, central corneal thickness measured by corneal pachymetry (measured in micrometers), and Pentacam (Oculus Optikgeräte GmbH, Wetzlar, Germany) used for corneal topography to map the surface curvature of the cornea.

ZNF469 and *PRDM5* Mutation Analysis

Peripheral blood samples were collected after informed signed consent. Genomic DNA was extracted according to the manufacturer's standard protocol (Qiagen miniprep; Qiagen, Venlo, The Netherlands). The coding exons and intronic splice junctions of *PRDM5* and *ZNF469* genes were amplified using primer pairs, published in previous studies, by polymerase chain reaction,[8,10] followed by Sanger sequencing for the proband of the family. Sequences obtained were aligned with

Received for publication August 11, 2017; accepted October 30, 2018. Published online ahead of print March 14, 2019.

From the *Department of Clinical Genetics, Academic Medical Centre, Amsterdam, The Netherlands; †Department of Pediatric Ophthalmology, Al-Shifa Eye Trust Hospital Jhelum Road, Rawalpindi, Pakistan; and ‡Department of Human Genetics, Radboud University Medical Centre, Nijmegen, the Netherlands.

The authors have no funding or conflicts of interest to disclose.

Correspondence: Arthur A. B. Bergen, PhD, Department of Clinical Genetics, Academic Medical Centre, Meibergdreef 9, Amsterdam 1105 AZ, the Netherlands (e-mail: aabergen@amc.uva.nl).

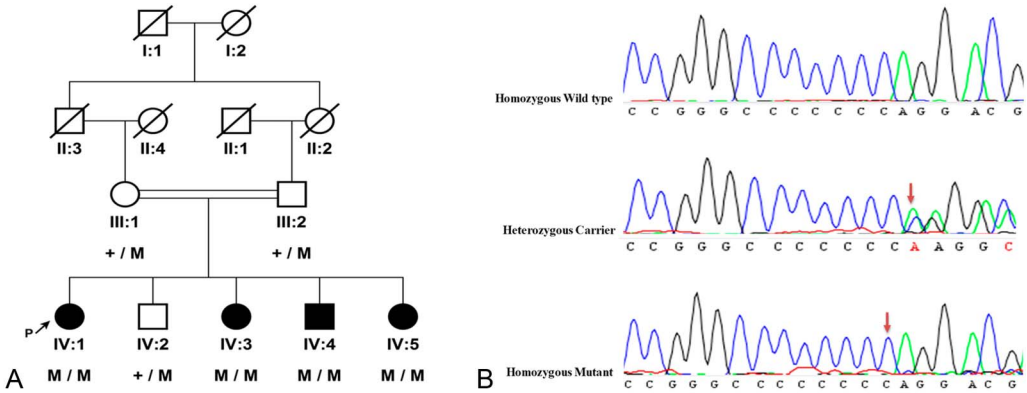

FIGURE 1. A, Segregation of genetic variant in a family with an autosomal recessive BCS: *ZNF469* c.9831dupC (p.Arg3278GlnfsX197). The proband is indicated with an arrow. Males are indicated with a square symbol and females with a circle; the affected individuals are indicated with filled black symbols. B, DNA sequence chromatogram of the mutation in *ZNF469*, which shows the homozygous wild-type sequence of control, heterozygous unaffected carrier, and homozygous affected individual. This mutation is co-segregating with the disease phenotype within the family. The downward arrow indicates the mutated nucleotide.

the reference sequence of *PRDM5* (NM-018699.3) and *ZNF469* (NM-001127464.2) using CodonCode Aligner (version 6.1). Intrafamilial segregation analysis was performed on the identification of DNA variant in the *ZNF469* gene in the proband.

RESULTS

Clinical Features and Family History

Four affected individuals of the family comprised of 3 females and one male underwent a detailed clinical examination for ophthalmologic and systemic abnormalities. The proband IV:1 (Fig. 1A) was a 10-year-old girl who presented with counting finger vision in both eyes. She had a congenital blue sclera in both eyes, with thin corneas. Her left eye was phthisical (Fig. 2A). She had a first corneal injury and surgery when she was 3 years old, and afterward she went through many surgeries because of corneal ruptures. She also had myopia with chorioretinal and peripapillary atrophy in the right eye. The cup disc ratio was 0.4, and a low intraocular pressure of 10 mm Hg was noted in right eye.

Sibling IV:3 (Fig. 1A) was a 6-year-old girl with a vision of 6/15 in both eyes. She also had blue sclera and nystagmus. She too underwent surgery of the right eye because of corneal rupture. Affected individual IV:4 was a 4-year-old male who presented with a trauma of the left eye while playing at home. He had blue sclera and nystagmus. He too went through surgery for corneal repair.

Affected individual IV:5 (Fig. 1A) was a 2-year-old girl with blue sclera. She had a thin cornea with keratoconus, with central corneal thickness measurement of 257 μm (normal range, 515–575 μm), progressive myopia, irregular astigmatism, and eventually marked reduction in visual acuity. The maximum keratometry values were 52.33 and 52.98 D for the right and left eye, respectively. Figure 3 shows the corneal topography maps for one affected individual (IV:5) and one unaffected individual (III:2) of the family.

None of the patients displayed any cardiovascular system abnormalities on echocardiography. By contrast, all of them had skeletal system abnormalities such as tall stature, long limbs, joint hypermobility, long narrow head, and flat feet (Fig. 2B, C).

Clinical features of all affected and unaffected family members are given in Table 1.

Mutation Detection

Sanger sequencing of the proband IV:1 born from consanguineous parents revealed a novel homozygous duplication

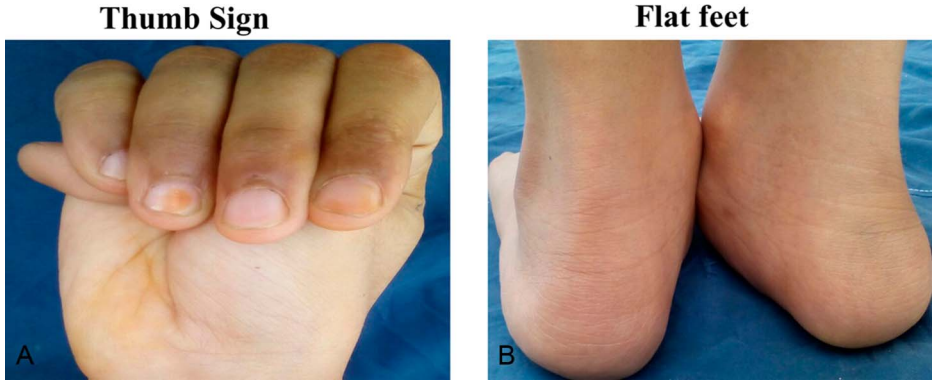

FIGURE 2. Hyperextensibility of joints. A, Thumb (STEINBERG) sign in which thumb is protruding out and is visible medial to the little finger. B, Flat feet with no arches under the feet.

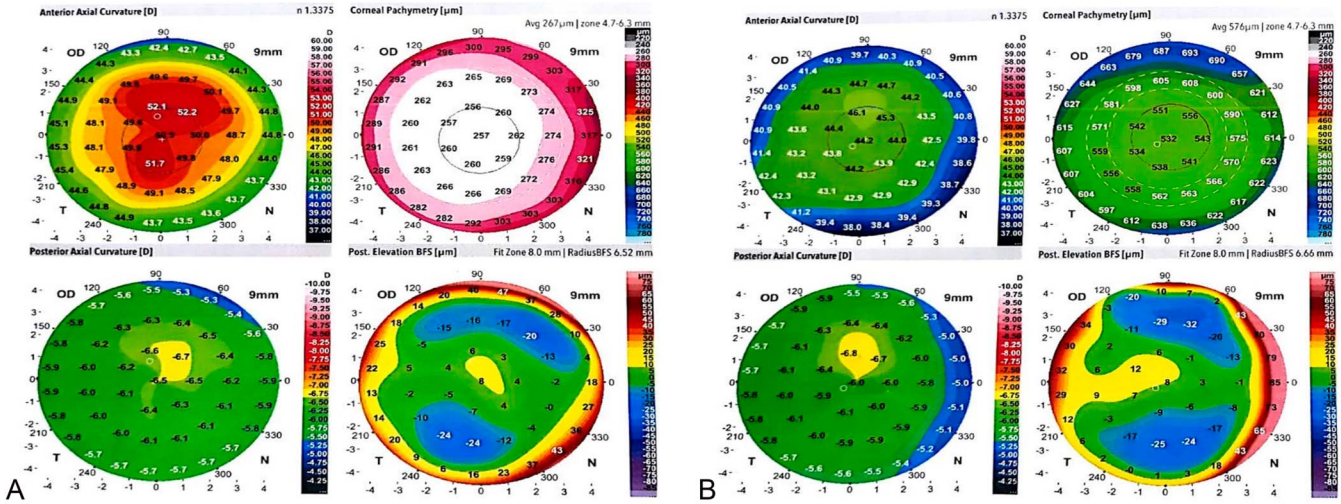

FIGURE 3. Axial curvature and Corneal pachymetry of affected and unaffected individuals. A, Data of the affected individual (IV:5) data. B, Data of the unaffected individual (III:2). In both (A, B), the left upper map shows the anterior axial instantaneous curvature (or tangential curvature), the left lower map shows the posterior axial curvature, the right upper map shows corneal thickness measured by corneal pachymetry, and the right lower map shows the best fit sphere eye surface in terms of micrometer.

(c.9831dupC; p.Arg3278Glnfs*197) in the *ZNF469* gene (Fig. 1B). This particular variant was co-segregating with the disease phenotype within the family. As expected, the affected individuals (IV:1, IV:3, IV:4, and IV:5) were homozygous for the duplication, whereas the unaffected parents (III:1 and III:2) and the sibling (IV:2) were heterozygous carriers. The c.9831dupC is absent in the Exome Aggregation Consortium and Genome Aggregation Database. All additional variants identified in *ZNF469* in the proband are listed in Table 2. Segregation analysis was performed for all missense variants identified in *ZNF469* gene with MAF < 1, but none of them co-segregated with the disease. In *PRDM5* gene, only 2 intronic single nucleotide polymorphisms (SNPs) (rs2136998 and rs756517674) were identified in the proband; however, no potential disease-associated variants were revealed.

TABLE 1. Clinical Features of Unaffected and Affected Family Members With Brittle Cornea Syndrome

	Affected individuals				Unaffected individuals		
Variable	**IV:1**	**IV:3**	**IV:4**	**IV:5**	**III:1**	**III:2**	**IV:2**
Current age (yr)	10	6	4	2	37	40	11
Gender	F	F	M	F	F	M	M
Eye							
Corneal perforation	+	+	+	+	−	−	−
Blue sclera	+	+	+	+	−	−	−
Myopia	+	+	+	+	+	+	−
Nystagmus	−	+	+	+	−	−	−
Keratoconus	−	−	−	+	−	−	−
Corneal thickness measured by pachymetry (µm)	280	263	259	257	489	532	494
Kmax for RE, D	50.37	44.30	48.19	52.33	44.53	46.76	44.68
Kmax for LE, D	NP	45.65	47.55	52.70	44.89	46.24	44.65
Astigmatism	2.89D	2.40D	2.30D	2.39D	1.37D	1.23D	1.32D
Soft or hyperelastic skin	+	+	+	+	−	−	−
Joint hypermobility	+	+	+	+	−	−	+
Hearing defect	−	−	−	−	−	−	−
Cardiovascular abnormalities	−	−	−	−	−	−	−
Skeletal system							
Arachnodactyly	+	+	+	+	−	−	−
Hyperextensible (fingers, wrist)	+	+	+	+	−	−	−
Flat feet	+	+	+	+	−	−	−

D, diopters; F, female; Kmax, maximum keratometry; LE, left eye; M, male; NP, not possible; RE, right eye.

TABLE 2. Variants Identified in *ZNF469* Gene in Proband

Nucleotide Changes in *ZNF469*	Amino Acid Change	Zygosity	Segregation Analysis	dbSNP	gnomAD SNP Frequency
c.1069T>C	p.Ser357Pro	Homozygous	Not segregating	rs11648572	0.9742
c.1098A>C	p.Arg366Ser	Homozygous	Not segregating	rs11640794	0.9151
c.1529 G>C	p.Gly510Ala	Homozygous	Not segregating	rs7199961	0.9987
c.3153T>C	p.= (p.Ile1051Ile)	Homozygous	Not segregating	rs9924504	0.9999
c.3484A>G	p.Lys1162Glu	Homozygous	Not segregating	rs7197071	0.8902
c.4259C>T	p.Pro1420Leu	Homozygous	Not segregating	rs4782300	0.8917
c.4335T>G	p.= (p.Ser1445Ser)	Homozygous	Not segregating	rs12445417	0.9358
c.5577C>G	p.= (p.Thr1859Thr)	Homozygous	Not segregating	rs9931465	0.8789
c.8520C>T	p.= (p.Arg2840Arg)	Homozygous	Not segregating	rs3812953	0.4893
c.8543A>G	p.His2848Arg	Homozygous	Not segregating	rs1983014	0.9988

gnomAD, Genome Aggregation Database.

DISCUSSION

In the current study, a novel duplication mutation c.9831dupC (p.Arg3278GlnfsX197) creating a frame shift starting at codon Arg3278 segregated with the BCS phenotype. The new reading frame ends in a STOP codon 196 positions downstream, which probably invokes nonsense-mediated mRNA decay. Figure 3 indicates that up to 3 zinc finger domains are lost because of this truncating mutation, which may lead to dysfunction of the encoded protein. In consanguineous families of diverse origin, which includes Jewish families of Tunisian origin, Palestinian,[4] Norwegian,[5] Syrian,[10] Arabic,[8] and Indian families,[11] mutations have been reported in *ZNF469* gene (Fig. 4). However, the British white girl having a *ZNF469* compound heterozygous mutation c.5788delC (p. Gln1930Argfs*6)/c. 5788dupC (p.Gln1930Profs*133) was born because of in vitro fertilization from unrelated parents.[11] So far, no Pakistani families with BCS have been reported with the causative variants in *ZNF469*. The current study is the first reporting a *ZNF469* pathogenic duplication in a Pakistani family.

In the Pakistani family with BCS, the affected individuals presented with both ocular and extraocular features; however, in our patients, glaucoma, hearing impairment, and cardiovascular abnormalities were not observed. Previously, in a Saudi Arabian family with c.4174G>T (p.Glu1392X) mutation in *ZNF469*, one of the ophthalmic phenotypes of affected individuals was congenital glaucoma.[10] Similarly, in another study, a 37-year-old Saudi Arabian male individual having a single base deletion mutation c.2149delT (p.Phe717Ser*fs*X15) had congenital glaucoma.[12] Interestingly, this individual did not have any of the extraocular abnormalities observed in other individuals presenting with BCS and *ZNF469* mutations. These findings indicate that BCS is clinically a heterogeneous disease and the diagnosis is complex. Similarly, clinical heterogeneity for disease was observed in one of our Pakistani family with BCS reported previously with *PRDM5* mutation. Initially, the family was diagnosed with Marfan syndrome, and on further identification of a homozygous *PRDM5* mutation c.93+5G>A, reexamination of the family revealed it as a family with BCS.[13] In a Pakistani family with *PRDM5* mutation, 2 of the affected individuals also had ectopia lentis,[13] which was not observed in other patients with either *PRDM5* or *ZNF469* mutation.

The *ZNF469* gene (NM_001127464) encodes a zinc finger protein of 3925 amino acids. The exact role of *ZNF469*

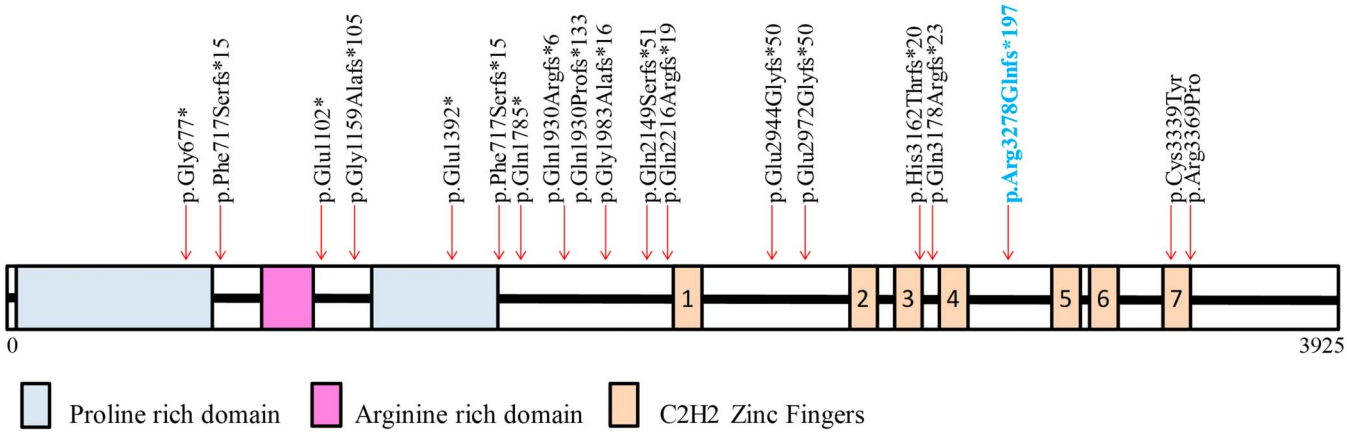

FIGURE 4. Schematic representation of *ZNF469* protein domains, with location of all mutations identified in families and patients with BCS, so far.

is not known. A number of genetic studies suggest its role in corneal development and proposed that *ZNF469* is a determinant of corneal thickness.[14,15] Indeed, Burkitt Wright and coworkers suggested that both *ZNF469* and the other BCS disease gene, *PRDM5*, regulate the turnover of the extracellular matrix (ECM) through the same molecular pathway. Genome-wide expression analysis of *ZNF469* and *PRDM5* mutant fibroblasts showed, independently, downregulation of multiple unique genes (*COL4A1*, *COL11A1*, *EDIL3*, *HAPLN1*, and *TGFβ2*) involved in ECM development and maintenance.[8] Furthermore, disorganization of other ECM members such as collagens I and III, fibronectin and their receptor α2β1 and α5β1 integrins was observed in fibroblasts of patients having mutations in *PRDM5* and *ZNF469*, which further supported the hypothesis that both interrupt the same biochemical pathway to cause the BCS phenotype.[8]

In summary, the results of this study broaden the mutation spectrum of *ZNF469* gene mutations in the families with BCS with different ethnicity which corroborates the role of *ZNF469* in the maintenance of corneal integrity.

REFERENCES

1. Zlotogora J, BenEzra D, Cohen T, et al. Syndrome of brittle cornea, blue sclera, and joint hyperextensibility. *Am J Med Genet.* 1990;36:269–272.
2. Burkitt Wright EM, Porter LF, Spencer HL, et al. Brittle cornea syndrome: recognition, molecular diagnosis and management. *Orphanet J Rare Dis.* 2013;8:68.
3. Al-Hussain H, Zeisberger SM, Huber PR, et al. Brittle cornea syndrome and its delineation from the kyphoscoliotic type of Ehlers-Danlos syndrome (EDS VI): report on 23 patients and review of the literature. *Am J Med Genet A.* 2004;124A:28–34.
4. Abu A, Frydman M, Marek D, et al. Deleterious mutations in the zinc-finger 469 gene cause brittle cornea syndrome. *Am J Hum Genet.* 2008; 82:1217–1222.
5. Christensen AE, Knappskog PM, Midtbo M, et al. Brittle cornea syndrome associated with a missense mutation in the zinc-finger 469 gene. *Invest Ophthalmol Vis Sci.* 2010;51:47–52.
6. Al-Owain M, Al-Dosari MS, Sunker A, et al. Identification of a novel ZNF469 mutation in a large family with Ehlers-Danlos phenotype. *Gene.* 2012;511:447–450.
7. Aldahmesh MA, Mohamed JY, Alkuraya FS. A novel mutation in PRDM5 in brittle cornea syndrome. *Clin Genet.* 2012;81:198–199.
8. Burkitt Wright EMM, Spencer HL, Daly SB, et al. Mutations in PRDM5 in brittle cornea syndrome identify a pathway regulating extracellular matrix development and maintenance. *Am J Hum Genet.* 2011;88: 767–777.
9. Hohenauer T, Moore AW. The Prdm family: expanding roles in stem cells and development. *Development.* 2012;139:2267–2282.
10. Khan AO, Aldahmesh MA, Mohamed JN, et al. Blue sclera with and without corneal fragility (brittle cornea syndrome) in a consanguineous family harboring ZNF469 mutation (p.E1392X). *Arch Ophthalmol.* 2010;128:1376–1379.
11. Rohrbach M, Spencer HL, Porter LF, et al. ZNF469 frequently mutated in the brittle cornea syndrome (BCS) is a single exon gene possibly regulating the expression of several extracellular matrix components. *Mol Genet Metab.* 2013;109:289–295.
12. Khan AO, Aldahmesh MA, Alkuraya FS. Brittle cornea without clinically-evident extraocular findings in an adult harboring a novel homozygous ZNF469 mutation. *Ophthalmic Genet.* 2012;33:257–259.
13. Micheal S, Khan MI, Islam F, et al. Identification of mutations in the PRDM5 gene in brittle cornea syndrome. *Cornea.* 2016;35:853–859.
14. Lu Y, Dimasi DP, Hysi PG, et al. Common genetic variants near the Brittle Cornea Syndrome locus ZNF469 influence the blinding disease risk factor central corneal thickness. *PLoS Genet.* 2010;6:e1000947.
15. Lechner J, Porter LF, Rice A, et al. Enrichment of pathogenic alleles in the brittle cornea gene, ZNF469, in keratoconus. *Hum Mol Genet.* 2014; 23:5527–5535.